Docket No.: P2028-702920

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	James A. Laugharn, Jr., et al.
Serial No.:	10/770,241
Filed:	February 2, 2004
Confirmation No.:	9463
Title:	RAPID CRYOBARIC STERILIZATION AND VACCINE PREPARATION
Examiner:	Elizabeth L. McKane
Art Unit:	1797

CERTIFICATE OF TRANSMISSION UNDER 37 C.F.R. § 1.8(a)

The undersigned hereby certifies that this document is being electronically filed in accordance with § 1.6(a)(4), on the 24th day of August, 2009.

/elias domingo/
Elias Domingo

Mail Stop Appeal Brief—Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPELLANTS' REPLY BRIEF PURSUANT TO 37 C.F.R. § 41.41

This Reply Brief is submitted in response to the Examiner's Answer mailed June 24, 2009 in the above-referenced patent application.

Table of Contents

I. Real Party in Interest (37 C.F.R. § 41.37(c)(i))

Pressure Biosciences, Inc. is the Real Party in Interest and was formerly known as Boston Biomedica, Inc. including its wholly owned subsidiary BBI Bioseq, Inc.

II. Related Appeals and Interferences (37 C.F.R. § 41.37(c)(ii))

There are no prior or pending appeals, interferences, or judicial proceedings related to the present application.

III. Status of Claims (37 C.F.R. § 41.37(c)(iii))

Claims 1-31 were originally filed. Claims 32-37 were added in a Response filed July 5, 2005. Claims 3-5, 8, and 15-31 were previously canceled.

Claims 1, 2, 6, 7, 9-14, and 32-37 are pending with claim 1 being the only pending independent claim.

All of the pending claims were rejected under 35 U.S.C. § 103(a) as would have been obvious over the disclosure of Hashizume *et al.*, in "Kinetic Analysis of Yeast Inactivation by High Pressure Treatment at Low Temperatures" (hereinafter "Hashizume") in view of the disclosure of Hayakawa *et al.*, in "Oscillatory Compared with Continuous High Pressure Sterilization on Bacillus stearothermophilus Spores" (hereinafter "Hayakawa").

The rejection of claims 1, 2, 6, 7, 9-14, and 32-37 is appealed.

A copy of the claims is attached as a Claims Appendix, beginning on page 17.

IV. Status of Amendments (37 C.F.R. § 41.37(c)(iv))

Claims 7, 32, 33, 36, and 37 were previously amended to correct typographical errors without adding new subject matter.

V. Summary of Claimed Subject Matter (37 C.F.R. § 41.37(c)(v))

The claims recite methods of sterilizing a material. (See *e.g.*, Specification at page 3, lines 23-26.) The methods include providing the material at an initial pressure; and while maintaining the material in a temperature range below 45 °C, (see *e.g.*, Specification at page 3, line 23 to page 4, line 1), increasing the pressure to an elevated pressure; and then decreasing the pressure to below the elevated pressure; and cycling the pressure between a decreased pressure and the elevated pressure at least two times to provide a sterilized material. (See *e.g.*, Specification at page 5, lines 1-5.)

Independent claim 1 is directed to a method for sterilizing a material. (See, for example, Specification at page 3, lines 23-26.) The method typically comprises providing said material at an initial pressure; and while maintaining said material in a temperature range that is below 45 °C (see, for example, Specification at page 3, lines 26-31, at page 6, lines 4-29, and the Examples), increasing the pressure to an elevated pressure (see, for example, Specification at page 4, lines 23-26, at page 6, line 13 to page 7, line 12, at page 11, lines 24-30, at page 15, lines 22-28, at page 16, lines 7-19, at page 25, lines 15 *et seq.*, and the Examples); then decreasing the pressure to below the elevated pressure (see, for example, Specification at page 3, lines 25-27, at page 5, lines 1-12, at page 7, lines 10-11, at page 8, line 30 to page 9, line 3, at page 10, lines 1-2 and lines 18-19, and at Examples); and cycling the pressure between a decreased pressure and the

elevated pressure at least two times, thereby providing a sterilized material (see, for example, Specification at page 7, lines 19 *et seq.*, at page 10, lines 20-24, at page 12, lines 10 *et seq.*, at page 13, lines 19 *et seq.*, at page 16, lines 26 *et seq.*, and at Examples).

VI. Grounds of Rejection to be Reviewed on Appeal (37 C.F.R. § 41.37(c)(vi))

A. Whether claims 1, 2, 6, 7, 9-14, and 32-37 would have been obvious over the disclosure of Hashizume in view of the disclosure of Hayakawa under 35 U.S.C. § 103(a).

VII. Argument (37 C.F.R. § 41.37(c)(vii))

A. Claims 1, 2, 6, 7, 9-14, and 32-37 would not have been obvious over the disclosure of Hashizume in view of the disclosure of Hayakawa

The pending claims recite methods of sterilizing a material by subjecting the material to cycled pressure (*i.e.*, cycling between elevated pressure and decreased pressure) and maintaining the temperature of the material below 45 °C.

Hashizume discloses methods of inactivating *Saccharomyces cerevisiae* (a strain of yeast), using high pressure treatment. Hashizume says that "rapid inactivation took place when the temperature was above 45 °C or below -10 °C." (See Hashizume page 1456, lines 17-19, left column.) Hashizume does not teach or suggest cycling pressure between an increased and decreased pressure, nor is Hashizume relied upon for such a teaching.

Hayakawa discloses examples of the use of oscillatory pressurization (*i.e.*, pressure cycling) to reduce the count of thermoduric spores of *Bacillus stearothermophilus*. Hayakawa tested the effects of oscillatory pressurization on spores, demonstrating that "(o)scillatory pressurization at 70 °C completely burst each spore along the length of the spore shape." (See Hayakawa, p. 165 and 166, RESULTS, Oscillatory pressurization, and Figure 5.)

When applying 35 U.S.C. § 103, the following tenets of patent law must be adhered to: (A) the claimed invention must be considered as a whole; (B) The references must be considered as a whole and must suggest the desirability and thus the obviousness of making the combination; (C) The references must be viewed without the benefit of impermissible hindsight vision afforded by the claimed invention; and (D) Reasonable expectation of success is the standard with which obviousness is determined. Hodosh v. Block Drug Co., Inc., 786 F.2d 1136, 1143 n.5, 229 USPQ 182, 187 n.5 (Fed. Cir. 1986).

The claimed subject matter is directed to methods of sterilizing material at temperatures below 45 °C using cycled pressure. The references, when considered as a whole, do not fairly teach the claimed methods. The Examiner cites Hayakawa as the only example of using cycled pressure, and yet the temperatures used in Hayakawa fall outside the claimed ranges. Moreover, as demonstrated in Figure 3 of Hayakawa, increasing the temperature to 70 °C with oscillatory pressure led to a greater reduction in survivors at two different pressures (*i.e.*, 400 MPa and 600 MPa).

Without the benefit of impermissible hindsight afforded by the presently claimed invention, one would not have arrived at methods for sterilizing material at temperatures below 45 °C using cycled pressure. Instead, one would have been motivated to use the methods clearly taught by Hayakawa. While the Examiner asserts that one would have been motivated to modify the teachings of Hashizume with Hayakawa to arrive at the claimed invention, the references, when taken as a whole, more fairly suggest methods of oscillatory pressure at elevated temperatures, *i.e.*, temperatures greater than 45 °C, such as 60 °C or 70 °C. Moreover, nothing in the combined references provide an expectation

of success when practicing the claimed invention. Rather, Hashizume notes that "No or little inactivation was observed for the pressurization below 180 MPa at temperatures between 0 °C and 40 °C." (See Hashizume page 1456, lines 17-19, left column.)

Hashizume also notes that "the pressure sterilization conditions can be improved further when sterilization is done at low temperatures including sub-zero temperatures." (Hashizume at page 1457, right column, lines 20-22.) Hashizume also observes that different microorganisms exhibit different sterilization characteristics. (See Hashizume at page 1457, left column, lines 8-17.) Thus, Hashizume concludes that, in contrast to heat inactivation, "pressure inactivation of microorganisms may give multiple kinetic patterns depending on the strains of microorganisms." (Hashizume at page 1457, left column, lines 17-21, emphasis added.)

Hayakawa, in contrast, notes that oscillatory pressurization at 70 °C of thermoduric *Bacillus stearothermophilus* "completely burst each spore along the length of the spore shape." (Hayakawa at page 165, right column, "Oscillatory pressurization.") Hayakawa concludes that this phenomena "must have resulted from two reasons: (1) the adiabatic explosion velocity of spore cell walls and high pressure water upon release of high pressures, and (2) water permeability into the spore cell wall and spore protoplasm under high pressure were promoted by the rise in temperature (70°C)." (Hayakawa at the last paragraph in the right column of page 166, emphasis added.) Hayakawa further suggests that the elevated temperature reduced the viscosity and surface tension of water, which made oscillatory pressurization more effective. (Hayakawa at the top paragraph in the left column of page 167.) Thus, one skilled in the art, upon reading Hayakawa, would

have concluded that sterilization techniques utilizing oscillatory pressurization requires higher temperatures, *i.e.*, 70 °C.

In contrast, the presently claimed subject matter is directed to techniques that involve temperatures below 45 °C. Thus, Hayakawa teaches against the presently claimed subject matter.

One skilled in the art would not have relied on Hayakawa's teachings to modify those of Hashizume. As noted, Hashizume concludes that "pressure sterilization conditions can be improved further when sterilization is done at low temperatures including sub-zero temperatures." Clearly Hayakawa's elevated temperature teaches against Hashizume's low temperature. Thus, because one skilled in the art would not have combined these references, claim 1 would not have been obvious.

Hashizume also notes that sterilization necessarily leads to the destruction of the natural structure of biological molecules, *i.e.*, proteins and lipids. (See Hashizume at the second paragraph of the left column on page 1455 and at lines 22 *et seq.* on the left column on page 1457, noting the similarity between protein denaturing and pressure inactivation with respect to pressure.) Thus, Hashizume's sterilization technique cannot involve the preservation of biological activity of macromolecules (such as proteins, carbohydrates, and nutrients), as recited in claim 34.

Because Hayakawa notes that complete destruction of spores is noted after six cycles, one skilled in the art would not have been motivated to further perform pressure

cycling for at least ten cycles, as recited in claim 9. Even if one skilled in the art would have further studied the effect of the number of pressure cycles, more than routine experimentation would have been required because of the variability in results for different microorganisms, as noted by Hashizume.

Further, one skilled in the art would not have had a reasonable expectation that Hayakawa's oscillatory pressurization approach would have provided satisfactory sterilization results in Hashizume's techniques for any microorganism. As also noted, Hashizume cautions that different microorganisms exhibit different sterilization characteristics and one skilled in the art would not have expected, without undue experimentation, that simply applying the oscillatory technique of Hayakawa to the sterilization process of Hashizume would have provided satisfactory sterilization results. Stated another way, because Hashizume cautions about such variability, one skilled in the art would not have had any reasonable expectation that cyclic pressurization would have provided predictable inactivation of any microorganism, as alleged with respect to claim 36.

The Examiner's response, at page 7, states that because Hashizume teaches that the known use of high temperatures is undesirable due to deterioration of fresh taste and flavor (at such condition), one of ordinary skill in the art would allegedly not have been motivated towards the high temperatures as taught by Hayakawa. This assertion, however, plainly contradicts Hayakawa's conclusion. As noted above, Hayakawa expressly relies on the high temperatures, in contradiction of Hashizume, to promote

water permeability, for the destruction of spores. Thus, contrary to the Examiner's assertion, one skilled in the art would have understood Hayakawa to require the high temperature (70 °C) to reduce the viscosity and surface tension of water to penetrate the cell wall. (See Hayakawa at page 167, concluding that complete sterilization was achieved at 70 °C.) The alleged motivation is thus misplaced because one skilled in the art, upon reading Hayakawa, would have been led to utilizing high sterilization temperatures, which contradicts the alleged "taste and flavor" rationale and, notably, would not have resulted in the claimed subject matter.

Hayakawa allegedly teaches that, regardless of pressure and temperature, cyclic exposure to pressure improves microorganism inactivation. Hayakawa, however, notes that the "effect of continuous pressurization on sterilization for 60 min under 600 MPa at 70°C was similar to that of six times oscillatory pressurization under 400 MPa at 70°C." (Hayakawa at page 165, "Oscillatory pressurization.") Thus, Hayakawa notes that oscillatory pressurization provides no sterilization advantage. Hayakawa also notes that the temperature dependence on sterilization is notable at 70 °C, but not at 20 °C. (See Hayakawa at page 165, "Temperature dependence.") Thus, one skilled in the art, reading Hayakawa as a whole, would have concluded that temperature, rather than cyclic pressurization, leads to effective microorganism inactivation. Further, even if one skilled in the art combined Hashizume and Hayakawa, the resultant sterilization procedure would have involved, at best, oscillatory pressurization at 70 °C because Hayakawa states that there is no distinction between continuous sterilization and oscillatory pressurization,

and because the rate of microorganism inactivation is notable or significant at such a temperature.

B. Conclusion

For the reasons provided herein, the rejection is improper and should be reversed. Applicants/Appellants therefore request that the rejection be withdrawn, and issuance of a Notice of Allowance.

VIII. Claims Appendix (37 C.F.R. § 41.37(c)(vii))

The following listings of the claims includes a marked-up version, showing amendments, and a clean version, incorporating the amendments.

Listing of Claims

(Marked-Up version)

1. (Previously presented) A method for sterilizing a material, the method comprising:

providing said material at an initial pressure; and

while maintaining said material in a temperature range that is below 45 °C, increasing the pressure to an elevated pressure, then decreasing the pressure below the elevated pressure, and cycling the pressure between a decreased pressure and the elevated pressure at least two times, thereby providing a sterilized material.

2. (Original) The method of claim 1, wherein the material is provided at an initial pressure of about 1 atm.

3-5. (Canceled)

6. (Original) The method of claim 1, wherein the elevated pressure is in the range of about 5,000 psi to about 120,000 psi.

7. (Previously presented) The method of claim 1, wherein the material comprises a desired biomolecule selected from the group consisting of nucleic acids, proteins, lipids, carbohydrates, drugs, steroids, and nutrients.

8. (Canceled)

9. (Previously presented) The method of claim 1, wherein the pressure is cycled at least ten times.

10. (Previously presented) The method of claim 1, wherein the decreased pressure is half of the elevated pressure or less.

11. (Original) The method of claim 1, further comprising warming or cooling the material prior to the pressure-increasing step.

12. (Original) The method of claim 1, further comprising warming or cooling the material after the pressure-increasing step.

13. (Original) The method of claim 1, wherein the material being sterilized is selected from the group consisting of a biological sample; blood plasma, serum, or other plant, animal, or human tissue; feces; urine; sputum; medical or military equipment; a foodstuff; a pharmaceutical preparation; ascites; and a vaccine.

14. (Previously presented) The method of claim 1, wherein the material being sterilized is initially contaminated with at least one of a prion, a virus, a fungus, a protist, a nucleic acid, and a protein.

15-31. (Canceled)

32. (Previously presented) The method of claim 1 in which the sterilized material comprises a desired macromolecule.

33. (Previously presented) The method of claim 14 in which the sterilized material comprises a desired macromolecule.

34. (Previously presented) The method of claim 32 in which the desired macromolecule has a biological activity that is maintained in the sterilized material produced by the method.

35. (Previously presented) The method of claim 32 in which the macromolecule is a protein.

36. (Previously presented) The method of claim 32 in which the sterilized material comprises an infectious agent, which is a virus.

37. (Previously presented) The method of claim 1, wherein the elevated pressure is in a range of about 2,000 psi to about 120,000 psi.

Listing of Claims

(Clean Version)

1. A method for sterilizing a material, the method comprising:

providing said material at an initial pressure; and

while maintaining said material in a temperature range that is below 45 °C, increasing the pressure to an elevated pressure, then decreasing the pressure below the elevated pressure, and cycling the pressure between a decreased pressure and the elevated pressure at least two times, thereby providing a sterilized material.

2. The method of claim 1, wherein the material is provided at an initial pressure of about 1 atm.

6. The method of claim 1, wherein the elevated pressure is in the range of about 5,000 psi to about 120,000 psi.

7. The method of claim 1, wherein the material comprises a desired biomolecule selected from the group consisting of nucleic acids, proteins, lipids, carbohydrates, drugs, steroids, and nutrients.

9. The method of claim 1, wherein the pressure is cycled at least ten times.

10. The method of claim 1, wherein the decreased pressure is half of the elevated pressure or less.

11. The method of claim 1, further comprising warming or cooling the material prior to the pressure-increasing step.

12. The method of claim 1, further comprising warming or cooling the material after the pressure-increasing step.

13. The method of claim 1, wherein the material being sterilized is selected from the group consisting of a biological sample; blood plasma, serum, or other plant, animal, or human tissue; feces; urine; sputum; medical or military equipment; a foodstuff; a pharmaceutical preparation; ascites; and a vaccine.

14. The method of claim 1, wherein the material being sterilized is initially contaminated with at least one of a prion, a virus, a fungus, a protist, a nucleic acid, and a protein.

32. The method of claim 1 in which the sterilized material comprises a desired macromolecule.

33. The method of claim 14 in which the sterilized material comprises a desired macromolecule.

34. The method of claim 32 in which the desired macromolecule has a biological activity that is maintained in the sterilized material produced by the method.

35. The method of claim 32 in which the macromolecule is a protein.

36. The method of claim 32 in which the sterilized material comprises an infectious agent, which is a virus.

37. The method of claim 1, wherein the elevated pressure is in a range of about 2,000 psi to about 120,000 psi.

IX. Evidence Appendix (37 C.F.R. § 41.37(c)(xi))

None.

X. Related Proceedings Appendix

None.

XI. Conclusion

For the reasons provided, Appellants respectfully request reversal of the rejections and issuance of a Notice of Allowance.

Please apply any charges or credits to deposit account no. 50/2762 (reference P2028-702920).

Respectfully submitted,
James A. Laugharn, Jr., *et al.*,
Applicants

By: /elias domingo /
Elias Domingo, Reg. No. 52,827
LANDO & ANASTASI, LLP
Riverfront Office Park
One Main Street
Cambridge, MA 02142
Tel: (617) 395-7087
Fax: (617-395-7070
Attorney for Applicants

Dated: August 24, 2009